Microbial ecology of extreme environments: Antarctic yeasts and growth in substrate-limited habitats.

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FINAL REPORT

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NAGW-26 Microbial Ecology of Extreme Habitats: Antarctic Yeasts and growth in substrate-limited habitats.

Final Report: 1 Dec 79-31 Aug 84

This project grew out of Dr. W.V. Vishniac's investigations of the thesis (of others) that life could not exist on other planets of this solar system, since this planet includes uninhabitable areas. Certainly life as we know it is limited by the boiling point of water and by great extremes of pH. The Ross Desert of Antarctica is an extreme environment with neither of these problems, but an environment which supposedly exemplified the inability of gean life to cope with combined stresses-- of low temperature, aridity, and nutrient limitation. The apparent sterility or abiotic (presence only of non-growing organisms) nature of certain Ross Desert soils has turned out to have been the result of inadequate methods of investigation. I have developed more adequate methods and am now able to describe significant aspects of the Ross Desert soil ecosystem. This project commenced at the point of the following publications:

Vishniac, H.S. and W.P. Hempfling. 1979. Evidence of an indigenous microbiota (yeast) in the Dry Valleys of Antarctica. J. Gen. Microbiol. 112: 301-314.

Vishniac, H.S. and W. P. Hempfling. 1979. Cryptococcus vishniacii sp. nov., an Antarctic yeast. Internat. J. Syst. Bacteriol. 29: 153-158.

and has resulted in the following publications:

Baharaeen, S. and H.S. Vishniac. 1981. Budding morphology of a psychrophilic Cryptococcus and related species compared with Leucosporidium scottii. Mycologia 73: 618-633.

Baharaeen, S. and H.S. Vishniac. 1982. A fixation method for visualization of yeast ultrastructure in the electron microscope. Mycopathologia 77: 19-22.

Baharaeen, S. and H.S. Vishniac. 1982. Cryptococcus lupi sp. nov., an Antarctic Basidioblastomycete. Internat. J. Syst. Bacteriol. 32: 229-232.

Baharaeen, S., Bantle, J.A., and H.S. Vishniac. 1982. The evolution of Antarctic yeasts: DN A base composition and DNA:DNA homology. Canad. J. Microbiol. 28: 406-413.

Vishniac, H.S. and S. Baharaeen. 1982. Five new Basidioblastomycetous yeast species segregated from Cryptococcus vishniacii emend. auct., an Antarctic yeast species comprising four new varieties. Internat. J. Syst. Bacteriol. 32: 437-445.

Vishniac, H.S. 1983. An enation system for the isolation of Antarctic yeasts inhibited by conventional media. Canad. J. Microbiol. 29: 90-95.

Baharaeen, S., U. Melcher, and H.S. Vishniac. 1983. Complementary DNA-25S ribosomal RNA hybridization: An improved method for phylogenetic studies. Canad. J. Microbiol. 29: 546-551.

Baharaeen, S. and H.S. Vishniac. 1984. 25S ribosomal RNA homologies of basidiomycetous yeasts: taxonomic and phylogenetic implications. Canad. J. Microbiol. 30: 613-621.

Vishniac, H.S. 1985. Cryptococcus friedmannii, a new species of yeast from the Antarctic. Mycologia 77 (Jan-Feb): IN PRESS

Vishniac, H.S. Submitted to Int. J. Syst. Bacteriol. Cryptococcus socialis sp. nov. and Cryptococcus consortionis sp. nov., Antarctic Basidioblastomycetes.

Vishniac, H.S. Submitted to Antarctic Journal of the US. Yeast biomass in Ross Desert (dry valley) soils: evaluation of quantitation methods and sample transport effects.

as well as numerous presentations at local, national, and international meetings. Copies of published papers have been sent to you (the last published herewith). Unpublished work is summarized below.

Most of these publications are "systematic", that is, they deal with the description and classification of organisms. The main purpose of this project was not systematic; systematics was essential to describing the ecology of the Ross Desert. These systematic studies also provided, as a bonus, theoretical insight of greater current importance than any provided by the Ross Desert ecosystem itself. It became clear, for the first time, that ribosomal RNAs evolve at different rates and so cannot provide a time scale by which to measure evolutionary distance (Baharaeen and Vishniac, 1984).

An extreme environment is by definition one with a depauperate biota. While the Ross Desert is by no means homogeneous, the most exposed and arid habitats, soils in the unglaciated high valleys, do indeed contain a very sparse biota of low diversity. So sparse that the natives could easily be outnumbered by airborne exogenous microbes. Native biota must be capable of overwintering as well as growing in the high valley summer. Tourists may undergo a few divisions before contributing their enzymes and, ultimately, elements to the soil-- or may die before landing. The simplest way to demonstrate the indigenicity of a particular microbe is therefore to establish unique distribution; occurrence only in the habitat in question precludes foreign origin.

The discovery of the yeast Cryptococcus vishniacii sp nov in Dr. W.V. Vishniac's last soil samples from the Ross Desert provided the first demonstration that indigenous microbes do live in the soils of the unglaciated high valleys (Vishniac and Hempfling, 1979a,b). This yeast species (and similar species) have now been searched for in soils as nearly similar as could be found in the Colorado Rockies -- without success (Vishniac, 1983). The Cr. vishniacii complex (Vishniac and Baharaeen, 1982) is apparently unique to the Ross Desert.

At the time when Cr. vishniacii was first described, however, at least one zymologist of the Centraalbureau voor Schimmelcultures (Baarn) expressed doubts as to its validity and generic assignment.

His doubts probably arose from the somewhat chaotic state of blastobasidiomycetes(yeasts related to mushrooms rather than to baker's yeast) classification. The genera of this group were defined in terms which modern microbiology had long since discarded. The species of these genera may vary widely in biotype (physiological profile)-- variant biotypes were assigned to species on the insufficient grounds of physiological similarity. Under these circumstances, the impact of the demonstration of indigenicity and the value of Cr. vishniacii as a model in investigating microbial adaptation to the multiply stressed Ross Desert habitat were somewhat diminished. DNA-DNA homology was used to define the species of the Cr. vishniacii complex (Baharaeen et al., 1982); 25S rRNA-cDNA homology was developed (Bahareen et al., 1983) and applied to the problem of defining fungal genera (Baharaeen and Vishniac, 1984). The Cr. vishniacii complex is not only unique to the dry valleys, it probably evolved there (Baharaeen et al., 1982). The species of the complex, the varieties of the species, are more closely related to each other than to any other described yeast species (Baharaeen et al., 1982). The closest 25S rRNA homologies of this complex are with psychrophilic Cryptococcus spp of the Himalayas, rather than with the far more common (and more likely to be imported as aerobiota) Cr. spp of more temperate soils.

These yeasts can therefore be used as an indicator of the fertility of suitable Ross Desert soils-- in fact, are the only available indicator of the biomass indigenous to such soils. Although procaryotes and algae can be isolated from these soils (see earlier progress reports from this project), no isolate has yet been shown to be indigenous. (The new species of bacteria which were described by some investigators were not incorporated into the "Approved List" in the Inter. J. Syst. Bacteriol., presumeably because of defects which are obvious in their description.). It is entirely possible that some of these microbes do form part of the soil community; it is simply difficult or impossible to demonstrate that they do.

Before indigenous yeast populations can be used to estimate soil fertility, one must be able to count them. The use of biochemical methods of biomass estimation is precluded both by the very low population densities in Ross Desert soils and by their failure to distinguish between tourists and natives. The indigenous yeasts are not recoverable with conventional yeast media (which accounts for the failure of previous investigators to find them). Their recovery in Dr. Hempfling's laboratory depended upon an ingenious and complex system of enrichment ("cascade enrichment") which was neither quantitative nor suitable for field use. The development of appropriate media and methods exemplifies the learning spiral which characterizes the investigation of every novel habitat. Studies of the first isolates enabled me to modify media, to isolate additional biotypes from dry valley soils, the studies of which---- etc. The current status of this problem is reported in the MS submitted to the Antarctic Journal of the US. The soils of high unglaciated valleys in the Ross Desert probably contain about 1 microcolony of indigenous yeasts per gram of soil, though populations of the order of 10^2 g^{-1} may occur. The medium (M3C) used in this field study does not allow continued growth of at least one biotype ("SLO") which was isolated from it, and so probably does not allow quantitative recovery of this biotype.

The estimate of 1 microcolony of indigenous yeasts per gram of soil differs somewhat from that given in the AJUS report. There are at least two reasons (besides the unsuitability of the medium used for biotype "SLO") why this figure is approximate. I have examined over 700 Antarctic yeast isolates. The last few are not yet assigned to biotype. Most of biotypes 19 and 23 through 64 (plus "SLO" and unidentifiable Sterigmatomyces sp.) have not been characterized sufficiently to allow final assignment to either old or new species of basidiomycetous psychrophiles. Since non-psychrophiles and non-basidiomycetes are equally rare among these isolates, all but biotype 41 are possible indigenes. Biotype 41 was kindly identified by Dr. C.P. Kurtzmann as Debaryomyces hansenii, an ascomycete with temperature preferences which debar it from naturalization in the Antarctic. But referring to these yeasts as indigenes is expressing an informed guess which might be modified in several cases were funds available for additional systematic investigation of the type which has been done for the Cr. vishniacii complex described.

The other reason for caution is the heterogeneity of the Ross Desert. The Ross Desert contains high unglaciated valleys, high glaciated valleys, and low valleys receiving glacial drainage; it contains soils, rocks, and streams, and ponds and lakes. There is a body of physical and biological evidence that each of the items mentioned harbours distinct communities, adapted to different physico-chemical or different climatic conditions. The absence of indigenous yeasts from some soil samples from the high unglaciated valleys (included in the AJUS estimate) has turned out to reflect the presence of salts similar to those found in the soils of lower altitudes. Ph.D. candididate J. Klingler (employed as RA under the grant this summer, with research costs provided by the Agronomy Department of this University) examined this summer the major cation content (by atomic absorption spectrometry)of each of the sufficiently large soil samples remaining, while I attempted to enrich 5 g samples of selected soils in simulated in situ conditions. The correlation between Na^+ content and yeast recovery (by any method) is shown in Table 1. While sodium was not always the dominant cation, there was no correlation with Ca^{++}, K^+, or Mg^{++}; correlation with sodium reflected the correlation with total major cation content. The analyzed soils came from various altitudes, from valleys with (University Valley) and without glaciers (the bulk of samples), with (Taylor Valley creek sediment) and without (the bulk of samples) visible water. It is clear that the methods used are suitable for indicating biomass only in soils of low salinity, those soils typical of the unglaciated high valleys. These soils are without exception fertile, albeit at low levels, with some form of microbial life. The problems of life at high salinity do not preclude fertility (see the work of Dr. Canale-Parola's group, for example Miller and Leschine 1982, Abstract I 19, ASM annual meeting), but are left to others.

The soil ecosystem in the high unglaciated valleys is not necessarily incomplete-- incomplete in the sense that it lacks primary producers -- though the bulk of energy probably arrives in the form of airspora. I have isolated algae from several samples (see previous progress reports and Vishniac, 1983). The isolates so

Table 1

Recovery of yeasts from sites in the Ross Desert of Antarctica by Na^+ and total cation content of soil samples.

Na^+ less than 5 μA g^{-1} highest total 8.53 μA g^{-1}	Na^+ 5.8-22.22 μA g^{-1} total 8.2-33 μA g^{-1}	higher Na^+ and total cation content (to 144 μA)
A801-29a*,b	A801-8*	A801-25*
A812-20a*,b	" -30a*,b	" -28*
" -23a	A812-23b	A812-1*
" -24a	" -24b	" -22*
A823-1	A823-2*	A823-6*
A834-51b	" -3*	" -7* (= 6b)
" -59	" -4*	" -10*
" -60	" -11	
	A834-53	
70% recovery by soil sample; 88% recovery by site: 20% of samples with populations exceeding 100 microcolonies g^{-1}.	" -57	No yeasts recovered.
	" -63*	
	" -65a*,b*	
	" -66	
	47% of samples fertile; 54% of sites. To 24 mc g^{-1}.	

* no yeasts recovered from this soil sample by any method yet attempted. Simulated in situ enrichment of 5 g soil samples has not been attempted for samples A801-29a,8,30a,30b, 25, or 28; A812-20a or 22. This method did demonstrate the presence of yeasts in A834-66 (highest cation burden in middle column) but not in A823-65b or A812-1 (lowest cation burden in last column. The limits between the middle and last column were chosen to reflect these data. Other samples subjected to simulated in situ enrichment were A812-20b, A823-1, and A834-59 (not analyzed).

"a", "b" refer to distance from surface; a usually 0-1 or 1-2 cm from surface, b usually 2-3 cm from surface or below a sample.

far identified(through the courtesy of Dr. E.I. Friedmann)include a probable phycobiont from cryptoendolithic lichens of Antarctic rocks and soil algae common to other climes. But a couple of falling ascospores could support a yeast microcolony (Vishniac and Hempfling, 1979a)--and Chaetomium ascospores deposited in soil have given rise to colonies in sprinkle plates on numerous occasions in my laboratory. (This fungus produced no other form of spore and is hardly likely to grow in the Antarctic, since its vegetative form does not survive freezing. It did not produce mycelia during simulated in situ enrichment, but only when transferred to agar media.) It takes very little of any required nutrient to allow maintenance and growth at Ross Desert temperatures and population densities.

What does limit the growth of indigenes in Ross Desert soils? Temperature, water, sources of carbon and energy, probably the population density and diversity of the community, probably not the availability of nitrogen, undoubtedly not the availability of inorganic nutrients. Simulated in situ enrichment of typical (thought to be marginally fertile) high valley soils was necessarily carried out at 10°, the highest likely summer subsoil temperature (and the probable temperature during best growing conditions in cryptoendolithic communities according to the work of Dr. E.I. Friedmann's group). Experiments would otherwise have taken too long, and it is well known that temperature is rate-limiting for growth of all microbes. Four 5 gram subsamples of selected soils were placed in sterile test tubes (sealed with parafilm to allow gas exchange only) and sampled (sprinkle plates) at 0, 6, and 20 days after various additions. One set of subsamples received no addition--evidence of possible growth was noted only in an initially moist sample (A812-20b). (Bacteria, yeasts, filamentous fungi, and algae appeared on various sprinkle plates but in insufficient numbers to ensure that each 5 g subsample did contain an initial inoculum; the presumption of marginal fertility for the majority of the soil samples tested was based on other types of examination, in most cases lacking statistical accuracy. The amount of soil used was limited by my wish to carry out other experiments with these samples.) The addition of water alone (0.1-0.2 ml, well below field capacity which generally runs to about 0.35 ml/5 g of sandy soils)to a second set of 5 g subsamples allowed some growth (for example in A823-1; there was no noticeable added effect in A812-20b). The presence of antibiotics (0.75 mg streptomycin sulfate + 2.5 mg penicillin G) in that water resulted in a population explosion(in A812-20b) which could be attributed either to removal of bacterial competition (the reason for adding antibiotics before adding additional carbon and energy sources) or to the contribution of dead bacteria to the nutritional environment or to the use of these molecules or their derivatives as nutrients. Independent bacterial counts in the soil samples used ranged from less than 10^2 cfu g^{-1} to 2.3×10^4 g^{-1}. Bacterial biomass in A812-20b was of the order of 10 ng (8.1×10^3 cfu g^{-1}), which is non-negligible as a nutrient source. It is therefore not surprising that the addition of 1 mg of glucose to the water + antibiotic mixture added to the fourth set of subsamples produced

in A812-20b

only the same explosion, though of a biotype which had not been previously isolated from this site, from other sites in University Valley, or from the other subsamples of A812-20b used in this experiment and so far appears to be novel in my collection. This vast excess of glucose did allow greater growth than the addition of antibiotics alone in A834-66. It also produced an explosion of Penicillium sp. in A823-1 from, presumeably, an exogenous spore. "Presumeably" because altho Penicillium spp. are the commonest airspora contaminating suitable media in the colder parts of the US, the inability of the vegetative hyphae to survive freezing or freeze-drying would prevent their naturalization in the Antarctic. Alternaria sp., Aspergillus sp., Chaetomium spp., Rhizopus nigricans, and dematiatiaceous hyphomycetes (most common in these soils after more than one months incubation of sprinkle plates) were present in various subsamples, but gave no evidence of growth in the enrichment tubes.

The absence of any form of nitrogen available to eucaryotes, of growth factors, or elements other than nitrogen which are customarily supplied in inorganic form in laboratory media made no apparent difference in any in situ enrichment of established sample fertility. These deficiencies in enrichment were expected to be met by soil composition (and could have been in part compensated for by antibiotic addition). The soil samples with the lowest content of K^+, Ca^{++}, and Mg^{++} (in J. Klingler's analyses) are known to be fertile. Similar soils analyzed in Dr. Hempfling's laboratory and by others elsewhere are known to contain adequate phosphorous sources, and adequate nitrogen in the form of nitrate. Growth factor requirements were not expected in organisms living in communities of low diversity and density.

What are the adaptations of the indigenous yeasts of the Ross Desert peculiar to their extreme environment? The list of qualifications for life in the Ross Desert must include psychrophily, the ability to survive wet and dry freezing and long arid periods, and minimal nutritional demands on the habitat. Minimal nutritional demands implies oligotrophy, freedom from growth factor requirements, and the use of nitrate-N, the most abundant N-source in desert soils and an N-source which is reportedly largely abiogenic in such soils. None of these traits is unique to the Cr. vishniacii complex of yeasts (alone or in combination). Soil yeasts generally (typically Cryptococcus spp.), and yeasts from less stressed Antarctic sites-- Leucosporidium scottii specifically-- have maximal growth temperatures below 20-25°. My medical mycology class has isolated Rhodosporidium capitatum from Oklahoma soil (which rarely exceeds 27° shortly below the surface even in a normal OK summer). This isolate would not grow at room temperatures above 22°. Yeasts and many procaryotes will survive wet freezing poorly, dry freezing very well. Preservation in dry soil is nearly as effective as lyophilization for some bacteria and for many yeasts and sporulating deuteromycetous fungi. I keep my stock educational cultures of yeasts frozen wet from year to year, my educational deuts in dry soil cultures for a minimum of 5 years with great success and a minimum of effort. The microcolony count of the most fertile Ross Desert soil samples did not vary significantly over more than 2 years in -80° storage, nor did their diversity appear to vary on somewhat cursory examination. This examination did include a search for yeasts which did not belong to the Cr. vishniacii complex. They were there in roughly the same proportions.

The widespread occurrence of basidiomycetous yeasts with minimal nutritional demands should perhaps not have been stated quite so positively. Leucosporidium scottii is a yeast with no growth factor requirements, utilizing nitrate-N, which has been reported only from Antarctic sites better supplied with sources of carbon and energy than the high unglaciated valleys. I have in the past proposed experimental determination (in chemostat) of the ability of L. scottii to compete with Cr. vishniacii for limited substrate. These experiments were never completed. The experiments themselves lost out in the competition for time, funds, and the talents of available personnel for the overall project. They failed in part because they could be postponed, while opportunities (which could not be postponed) for the acquisition of other types of data presented themselves. Given the reports of oligotrophy in Rhodotorula (a yeast isolate of marine origin in this case) by Button and co-workers, and the implications of the alternatively acquired data, I believe that my decision was correct. Factors in addition to oligotrophy as such are probably important in the adaptation of yeasts to the substrate-limited dry valley soils. In the case of L. scottii, it might well have lost out because its common response to gradual substrate limitation is the production of mycelium, a response which would be fatal in Antarctica.

What does it take, then, to inhabit the most exposed parts of earth's coldest desert? Why are yeasts belonging to (or resembling) the Cr. vishniacii complex indigenous to the dry valleys, while the congeneric common soil yeasts of other areas were isolated only so rarely as to suggest that they traveled with the mesophilic Debaryomyces hansenii ? --while the basidiomycetous yeasts of less-stressed Antarctic sites have appeared totally absent from the high unglaciated valleys? Thanks to the kindness of Dr. E.I. Friedmann and his ACME (Antarctic Cryptoendolithic Microbial Ecosystem) group, I have been able to examine a number of soil samples from the seasons '80-'81 (A801) through '83-'84 (A834) which were transported frozen to my laboratory and (in propria persona) to examine the Ross Desert sites, collect samples, and enumerate the microbiota of most at McMurdo Station during part of the A834 season. When the distribution of isolate characters by site was analyzed, two additional factors (besides substrate-limitation) of high probable significance appeared. The analysis would be improved by further isolate characterization. Poor taxonomy produces poor ecological analysis.

The first factor to emerge was N-source, confirming the implied importance of nitrate-N utilization. Yeasts failing to utilize nitrate-N appeared in only 5 of 18 fertile soil samples, typically accompanied by nitrate-utilizers and microbial diversity suggesting a relatively high biogenic ammonia flux. Such yeasts therefore appear to be obligately "social" in Antarctica. The dominant (in the dry valleys) asocial yeasts require biogenic compounds only as substrates, not as sources of nitrogen or growth factors. A major adaptation is therefore to life in depauperate communities.

Some yeasts (exemplified by L. scottii and several Rhodosporidium/Rhodotorula spp.) which occur in more conventional habitats make equally few demands upon their environment. A reason for their exclusion from the high unglaciated valley soils is suggested by their distribution in Antarctica. We may exclude the Sporidialean yeasts. Leucosporidium and Rhodosporidium (with their Vanrija and Rhodotorula anamorphs) share a tendency to produce mycelium under substrate limitation, even when suitable dikaryons/diploid nuclei for sexual reproduction have not been acquired. The low probability of surviving freezing conditions when engaging in this activity has already been pointed out. The Filobasidiales (and their Cryptococcus anamorphs) produce mycelium only when reproducing sexually or undergoing selection pressure far in excess of that found in nature. (Eg. Cryptococcus neoformans deliberately fed to grazing amoebae.) The best counterpart among conventional yeasts for the Cr. vishniacii complex is the Cr. terreus-elinovii complex. This complex consists of at least two, probably more, species, less common in most soils than Cr. albidus and Cr. laurentii (and its segregants), with indistinguishable physiological profiles. The species Cr. terreus, as presently described, is poorly defined and difficult to identify positively.

The second factor which emerged, or rather, seems to be emerging, is the importance of sources of water other than occasional snowfall. Others have suggested that osmotolerance may not be an altogether adequate measure of microbial xerotolerance. The osmotolerance of Cr. terreus does not seem to be recorded (other than as failure to grow in 50% glucose). My isolates of ? Cr. terreus so far appear no more osmotolerant and no less osmotolerant than my isolates of the Cr. vishniacii complex and other asocial yeasts resembling them. But my isolates come and come only from soil samples from University Valley, a high valley with soil of low salinity, furnished with a small glacier at its head. These isolates are incompletely characterized, but would be identified by a hurried taxonomist (concerned mainly with tagging specimens) as Cr. terreus. I have not examined the type of this species. I have examined creek sediment of appropriate salinity from lower Taylor Valley, so moist that it reached me as a block of ice. It contained a variety of yeasts (largely Sporidialean), but was patently (from diatom tests, cyanobacteria etc. present) very different from other sites in respects other than water content.

I cannot close without expressing the wish that there were some source of funds(other than my princely salary) to complete the analysis of this ecosystem, if not of the mechanisms of adaptation. It is the most exposed, in an area unique on earth, example of adaptation to cold desert conditions. Gean life can and did adapt.

Nor can I close without again expressing my thanks to NASA for support, out of funds grudgingly granted by Congress, for the work which has been done, to my colleagues here and elsewhere (thanked in published works), students (especially the current crop now supporting themselves in teaching assistantships), technicians, and work/study students (most of whom have again been thanked in publications, others of whom will be shortly).